Complementary approaches to identify genes important for chromosome segregation

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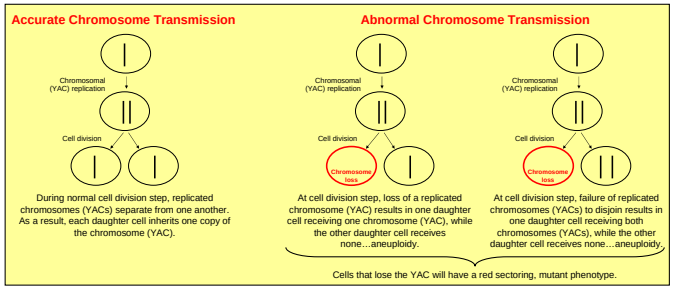
Abstract

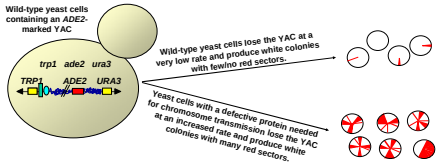
Faithful transmission of chromosomes during cell division is essential to the functioning of the eukaryotic cell. In humans, errors during chromosome segregation are correlated with diseases such as Down Syndrome and cancer. To elucidate the genes and processes required for accurate chromosome segregation, two types of genetic approaches using the model eukaryotic organism *Saccharomyces cervisiae* (baker's yeast) are being implemented. Both approaches rely on an assay system in which the segregation of a non-essential yeast artificial chromosome (YAC) is monitored. The first approach begins with the isolation of mutants with defective chromosome segregation and seeks to identify the gene(s) responsible for producing the mutant phenotype. Four previously isolated YAC stability in mitosis mutants (*ysm's 22, 77, 83,* and *84*) were characterized as having a marked increase in YAC loss. To identify genes that suppress the YAC loss phenotype in these mutant strains, the *ysm* mutants were transformed with a yeast genomic plasmid library and screened for plasmids that suppress the mutant phenotype. The suppressor candidates were re-screened, and the specific genes responsible for the suppression are currently being determined. The second approach begins with a known mutant or nonfunctional gene and seeks to discover the effect of this known mutation on chromosome segregation. Five genes of known function that are suspected to be important for accurate transmission of chromosome (*MRC1*, *MRE11*, *MUS81*, *RAD9*, and *RAD27*) are targeted in this analysis. Following deletion of these genes in a yeast strain containing a YAC, the loss rate of the YAC will be experimentally determined in the deletion mutants and compared to the loss rate in a wild-type strain. These investigations are expected to reveal genes important for the process of chromosome segregation.

Background

- **Which genes are important for chromosome transmission?** Our goal is to utilize forward and reverse genetic approaches to solve this question.
- **Why is chromosome transmission important?** Without accurate chromosome transmission, pathology such as aneuploidy (e.g., Down Syndrome and cancer) can result.
- **What causes abnormal chromosome transmission?** Incomplete DNA replication, nondisjunction, and failure of chromosomes to attach to the mitotic spindle during cell division can cause daughter cells to receive an abnormal number of chromosomes.

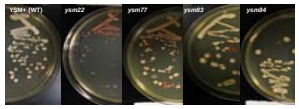


- **How do we detect chromosome loss?** Chromosome loss can be detected by monitoring the transmission of a yeast artificial chromosome (YAC) in the model eukaryote, *Saccharomyces cerevisiae* (baker's yeast). AHJ1-3-19B "wild-type" cells have a defective *ade2* gene which causes red colony color. However, if a YAC containing a functional *ADE2* gene is present in these cells, the colonies are white in color. Cells that lose the YAC at an increased rate appear white with red sectors.

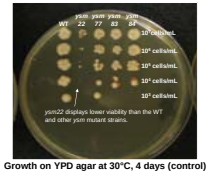


Forward Genetics Approach

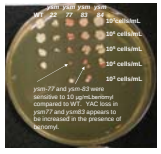
Phenotypes of *ysm* mutant strains



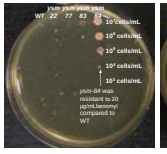
Sectoring phenotypes of WT (*YSM+*) and *ysm* mutants on YPD agar



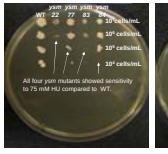
Growth on YPD agar at 30°C, 4 days (control)



YPD + 10μg/mL benomyl, Room temp., 7 days



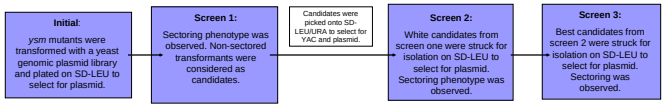
YPD + 20μg/mL benomyl, Room temp., 7 days



YPD + 75 mM hydroxyurea (HU), Room temp., 7 days

YPD + 5 min. ultraviolet (UV) light exposure

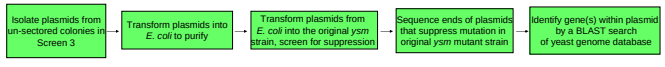
Genetic screen to isolate suppressors of the YAC loss phenotype of *ysm* mutants



Screens for Plasmid Suppression

	Number of Suppressor Candidates			
ysm strain	Initial	Screen 1	Screen 2	Screen 3
ysm22	6003	>300	8	8
ysm77	5366	200	47	6
ysm83	200	74	69	10
ysm84	444	175	84	16

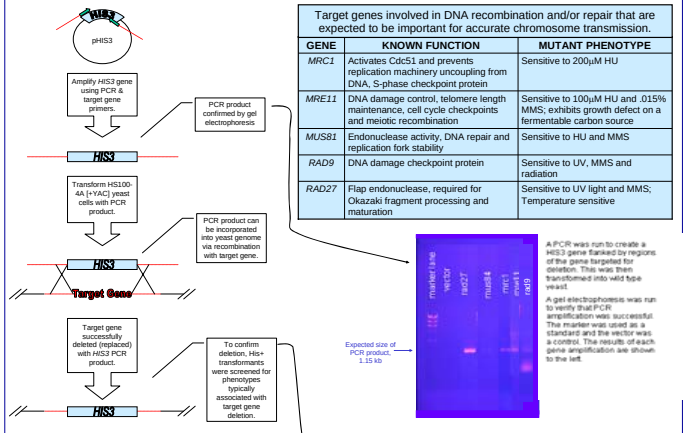
Analysis of Suppressor Plasmids



Conclusions and Future Work

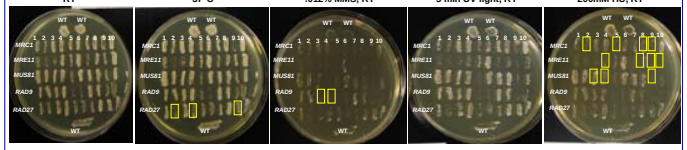
- *ysm77* and *ysm83* sensitivity to benomyl in a concentration of 10 μg/ml indicates that these supersensitive strains possess a mutation compromising microtubule stability and is consistent with benomyl's mechanism of action. In contrast, *ysm84* displays resistance to 20 μg/ml benomyl, a phenotype characteristic of tubulin mutants.
- Sensitivity to hydroxyurea in a concentration of 75 mM was observed in all *ysm* mutants tested. Sensitivity at this concentration suggests that these strains are predisposed to errors in replication.
- From the lack of sensitivity to ultraviolet light it can be inferred that (1) mutant strains are capable of DNA error correction when concerning proper cell division or that (2) the amount of time cells underwent UV exposure was insufficient to induce mutations that could be predicted visually.
- The low growth phenotype observed in *ysm22* suggests that the mutation incurred by this strain is important not only for chromosome transmission, but also for viability.
- Suppressor plasmids will be analyzed to determine the specific genes that suppress YAC loss in the *ysm* mutants.

Reverse Genetics Approach



Target genes involved in DNA recombination and/or repair that are expected to be important for accurate chromosome transmission.

GENE	KNOWN FUNCTION	MUTANT PHENOTYPE
MRC1	Activates Cdc51 and prevents replication machinery uncoupling from DNA, S-phase checkpoint protein	Sensitive to 200μM HU
MRE11	DNA damage control, telomere length maintenance, cell cycle checkpoints and meiotic recombination	Sensitive to 100μM HU and .015% MMS; exhibits growth defect on a fermentable carbon source
MUS81	Endonuclease activity, DNA repair and replication fork stability	Sensitive to HU and MMS
RAD9	DNA damage checkpoint protein	Sensitive to UV, MMS and radiation
RAD27	Flap endonuclease, required for Okazaki fragment processing and maturation	Sensitive to UV light and MMS; Temperature sensitive



Target gene deletion candidates were picked as patches to SD-HIS agar and replica plated with the following treatments: incubation at room temperature (RT) for 2 days, incubation at 37ºC for 2 days, YPD agar containing methyl methansulfonate (MMS) incubation at RT for 4 days, exposure to ultraviolet (UV) light for 5 min and incubation at RT for 2 days, YPD agar containing 200 mM hydroxyurea (HU) and incubation at RT for 4 days. Patches marked with yellow boxes are the best candidates for gene deletion based on phenotype.

Conclusions and Future Work

- A PCR strategy was used in an effort to delete specific target yeast genes (*MRC1*, *MRE11*, *MUS81*, *RAD9*, and *RAD27*) suspected to be important for accurate chromosome transmission. Candidate deletion strains were tested for phenotypes associated with the deletion strains.
- Following confirmation of the gene deletions, YAC loss in these deletion strains will be analyzed using both qualitative and quantitative assays.
  - Qualitative analysis: Wild-type and deletion strains will be single colony purified on rich media that allows for loss of the YAC. The sectoring phenotype of the deletion strains will be compared to wild-type cells. Increased sectoring in the deletion strain would indicate increased chromosome (YAC) loss.
  - Quantitative analysis: The actual YAC loss rates in the wild-type and mutant strains will be calculated using a comparative method that requires multiple generations of cells to be cultured and plated on YPD agar. The frequency of YAC loss is first calculated followed by its inclusion into an equation that will determine rate of loss. High loss rates relative to the wild-type strain would suggest that the deleted gene plays an essential role in the chromosome replication/segregation process.

Acknowledgements & References

- **We thank Dr. Brian Lenzmeier (Buena Vista University) for providing PCR primers and pHIS3 plasmid. We also thank Fall 2006 BIO106 (Research in Genetics) students who generated the *ysm* mutant collection.**
- Munich Information Centre for Protein Sequences (MIPS) *Saccharomyces cerevisiae* genome database. 2003. <www.mips.gsf.de/genre/proj/yeast>.
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